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חוק הפטנטים, תשכ"ז – 1967
ENTS LAW, 5727-1967

בקשה לפטנט
Application For Patent

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אני, (שם המבקש, מענו ולגבי גוף מאוגד ת מקום התאגדותו)
I, (Name and address of applicant, and in case of body corporate-place of incorporation)

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בעל אמצאה מכח | הדין | Owner, by virtue of

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חומרים להדמיה ושיטות איבחון המשתמשות בהם

Agents for imaging and diagnostic methods using them

(באנגלית)
(English)

Hereby apply for a patent to be granted to me in respect thereof.
מבקש בזאת כי ינתן לי עליה פטנט

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Appl. for Patent of Addition</td><td colspan="3">דרישת דין קדימה*
Priority Claim</td></tr>
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to Patent/Appl.</td><td>מספר/סימן
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Date</td><td>מדינת האיגוד
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Patent Attorneys
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<tr><td colspan="4">חתימת המבקש
Signature of Applicant
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ריינהולד כהן ושותפיו
ע"י :-</td><td colspan="3">היום This   28   of   בחודש   November   חודש   שנת 2002 Year

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חומרים להדמיה ושיטות איבחון המשתמשות בהם

Agents for imaging and diagnostic methods using them

NST Neuro Survival Technologies Ltd.

נסט ניורוסורוויל טכנולוגיס בע״מ

C. 142170

AGENTS FOR IMAGING AND DIAGNOSTIC METHODS USING THEM

FIELD OF THE INVENTION

The present invention relates to novel compounds useful as imaging agents, and diagnostic methods using them for detecting a disease process, for monitoring the progression of a disease and/or for monitoring of the effect of treatment.

5

LIST OF REFERENCES

The following references are considered to be pertinent for the purpose of understanding the background of the present invention:

Bevers, E.M., *et al., Biochim. Biophys. Acta,* **1439**:317-330, 1999;

10 Bombeli, T., *et al., Blood,* **89**:2429-2442, 1997;

Bratton, D.L., *et al., J. Biol. Chem.,* **272**:26159-26165, 1997;

Bursch, W., *et al., Trends Pharmacol. Sci.,* **13**:245-251, 1992;

Kockx M.M., *et al., Cardiovasc.Res.,* **45**:736-746, 2000;

Mallat, Z., *et al., Circulation,* **96**:424-428, 1997;

15 Martin, S., *et al., J. Exp.Med.,* 182:1545-1556, 1995;

Sims ,P.J., *et al., Thromb. Haemost.,* **86**:266-275, 2001;

Stary, H.C., *et al., Circulation,* **92**:1355-1374, 1995;

Van den Eijnde, S.M., *et al., Cell Death Diff.,* **4**:311-316, 1997.

20 The above references will be acknowledged in the text below by indicating in brackets, from the above list, the name of the first author and the year of publication.

BACKGROUND OF THE INVENTION

Cell membranes of intact eukaryotic cells are characterized by a highly organized structure. This high level of organization is determined, among others, by the molecular structure of the specific lipids constituting the membranes; the ratio between the various lipid species from which the membrane is composed; the distribution of the phospholipids between the outer and inner leaflets of the membranes; and by the protein components of the membrane.

While maintenance of the high level of membrane organization is fundamental to normal cell physiology, substantial perturbations and alterations of the normal organization of membrane (PNOM) occur in numerous physiological and pathological conditions, and are characterizing a plurality of diseases (Martin, S., et al., 1995). Such alterations and perturbations may be evident both at the morphological level (membrane blebbing observed in cells undergoing apoptosis) and at the molecular level. The scope of perturbations accompanying either cell activation, cell disease or cell death is not fully elucidated. They include, among others, scrambling and redistribution of the membrane phospholipids, with movement to the cell surface of aminophsopholipids, mainly phosphatidylserine (PS) and phosphatidylethanolamine (PE), which are normally restricted almost entirely to the inner leaflet of the membrane bilayer, and movement of sphingomyelin and phosphatidylcholine from the outer leaflet to the inner leaflet of the membrane (Sims, P.J., et al., 2001). This redistribution is referred herein as loss of cell membrane lipid asymmetry (CMLA). These alterations play an indispensable role in making the cell surface a catalytic platform for the assembly of several clotting factor complexes, such as tenase and prothrombinase complexes (Bevers, E.M., et al., 1999). Thus, platelets undergo PNOM upon activation, and these alterations constitute an important factor in normal blood coagulation, as well as in the initiation and/or propagation of abnormal, excessive blood clotting in numerous disorders. These disorders include, among others, arterial or venous thrombosis or thrombo-embolism [e.g., cerebral stroke, myocardial infarction, deep vein thrombosis (DVT), disseminated intravascular coagulation (DIC), thrombotic

thrombocytopenic purpura, etc.]; unstable atherosclerotic plaques, sickle cell disease; beta-thalassemia; anti-phospholipid antibody syndrome; among others in systemic lupus erythematosus (SLE); disorders associated with shedding of membrane microparticles, e.g., neurological dysfunction in association with

5 cardiopulmonary bypass.

Apoptosis is another major situation in which alterations/perturbations of cellular membranes take place (Bratton, D.L., *et al.*, 1997). Apoptosis is an intrinsic program of cell self- destruction or *"suicide"*, which is inherent in every eukaryotic cell. In response to a triggering stimulus, cells undergo a highly characteristic

10 cascade of events of cell shrinkage, blebbing of cell membranes, chromatin condensation and fragmentation, culminating in cell conversion to clusters of membrane-bound particles (apoptotic bodies), which are thereafter engulfed by macrophages (Bursch, W., *et al.*, 1992). PNOM is a universal phenomenon in apoptosis, it occurs early in the apoptotic cascade, probably at the point of cell

15 commitment to the death process, and has also been shown to be an important factor in the recognition and removal of apoptotic cells by macrophages (Van den Eijnde, S.M., *et al.*, 1997).

A strong correlation has been recently drawn between PNOM and potent procoagulant activity of apoptotic cells (Bombeli, T., *et al.*, 1997). PNOM in

20 apoptotic endothelial cells, such as in atherosclerotic plaques (Mallat, Z., *et al.*, 1997), probably plays an important role in the pathogenesis of thrombotic vascular disorders. PNOM is also a feature of inflammatory cells (i.e., lymphocytes, macrophages), activated by various triggers.
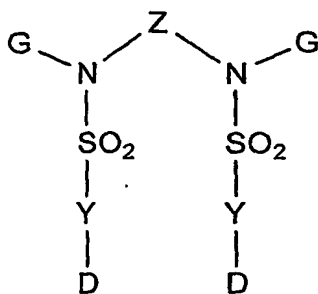
Since apoptosis, thrombosis or inflammation have an important role in the

25 majority of medical disorders, it is desirable to have tools for detection of these biological processes. Compounds for selective binding to PNOM membranes, potentially also performing subsequent entry into these cells having such PNOM membranes, may therefore serve as an important tool for detecting and targeting of cells undergoing activation, damage or death process, especially by apoptosis. In

30 the clinical context, detection of binding to said membranes may be useful in the

diagnosis of disease processes, in monitoring course or progression of a disease, or in monitoring the effect of various therapeutic approaches utilized to alter disease course.

5   It is an object of the present invention to provide new compounds and their uses as imaging agents for detecting a disease process, for monitoring the progression of a disease process, and / or for monitoring of the results of therapy.

According to one aspect, the present invention provides new sulfonamide compounds, having the following formula I:

10

$$
\begin{array}{c}
G{-}N{\overset{\displaystyle Z}{\diagup}}{\diagdown}N{-}G \\
\quad\ \ \, |\qquad\qquad\ \ | \\
\quad\ \ SO_2\qquad\quad\ SO_2 \\
\quad\ \ \, |\qquad\qquad\ \ | \\
\quad\ \ \, Y\qquad\qquad\ \ Y \\
\quad\ \ \, |\qquad\qquad\ \ | \\
\quad\ \ \, D\qquad\qquad\ \ D
\end{array}
$$

(I)

including pharmaceutically acceptable salts, metal chelates and hydrates of the structure of formula (I); wherein

15   Y groups may be the same or different, each being an aromatic, optionally heterocyclic, 8-10 atom, two-ring system;

G groups may be the same or different and are selected independently among hydrogen, $-(CH2)_mCH(NH_2)(COOH)$, $-(CH2)_m(COOH)$, $-(CH2)_mNH_2$; m being an integer of 0-4;

20   D is WR_b, wherein W is selected among null, N, O, S and C;

R represents hydrogen or a C_1-C_6 alkyl; R moieties may be either the same or different; and

b is 1, 2 or 3;

Z is selected from a group having the formula U(A)-T-U(A)- and a group having the formula -U-A-U-T-U-A-U-

U groups may be the same or different, and are each independently selected from null or an optionally substituted C_1-C_4 alkylene, C_2-C_4 alkenylene, C_3-C_8 branched alkylene, and C_3-C_8 branched alkenylene;

T is selected among -A-, -A(B)-, -(B)A(E)-, -Q-, -Q(B)-, and -A(B-Q)-;

wherein A groups may be the same or different, and are selected among null, hydrogen and ionizable groups; charge(s) of said groups being, upon ionization selected among positive charge(s), negative charge(s), and zwitterion forms; wherein at least one A group is other than null and the total charge of the compound is either negative or zwitterion;

B groups may be the same or different, and are selected from hydrogen or an optionally substituted C_1-C_6 alkyl, C_1-C_6 alkylene, C_2-C_6 alkenyl, C_2-C_6 alkenylene, C_3-C_8 branched alkyl, C_3-C_8 branched alkylene, C_3-C_8 branched alkenyl, C_3-C_8 branched alkenylene, aryl, heteroaryl and combinations thereof;

E is selected from null, hydrogen and a C_1-C_6 alkyl; and

Q is selected among null, a marker for imaging, or a metal chelator; said marker for imaging being selected from the group comprising a fluorescent label, a radio-label, a marker for X-ray, a marker for MRI, or a label capable of undergoing an enzymatic reaction that produces a detectable color.

In a preferred embodiment, Q is a metal chelator. Advantageously, Q forms metal chelates with Technetium, oxo-technetium, Gallium, Rhenium or oxo-rhenium isotopes.

In another preferred embodiment Q is selected among ^{18}F, ^{13}C and ^{124}I. In such case, Q may also be linked directly to the Y or D moieties.

In another preferred embodiment U groups are each independently selected among null and an optionally substituted C_1-C_3 alkylene group; D is NR_2, wherein each R group is selected independently from hydrogen and C_1-C_4 alkyl;

In yet another preferred embodiment, A comprises acidic group(s) selected from carboxylic, phosphoric, phosphatic, sulfonic and sulfuric acid;

In the case that Q is a metal chelator, said chelator preferably comprises nitrogen, sulfur and / or oxygen atoms participating in metal chelation. In such preferred embodiment, metal chelation is accomplished through a combination of atoms selected from three nitrogen atoms and one sulfur atom; two nitrogen atoms and two sulfur atoms; or one nitrogen atom and three sulfur atoms. Examples of such metal chelators are chelators comprising diaminedithiols, monoamine-monoamide-bisthiols (MAMA), triamide-monothiols, and monoamine-diamide-monothiols, e.g., metal chelators comprising N_2S_2, NS_3, and N_3S atoms.

In a preferred embodiment the compounds of the present invention have the following formula II:

(II)
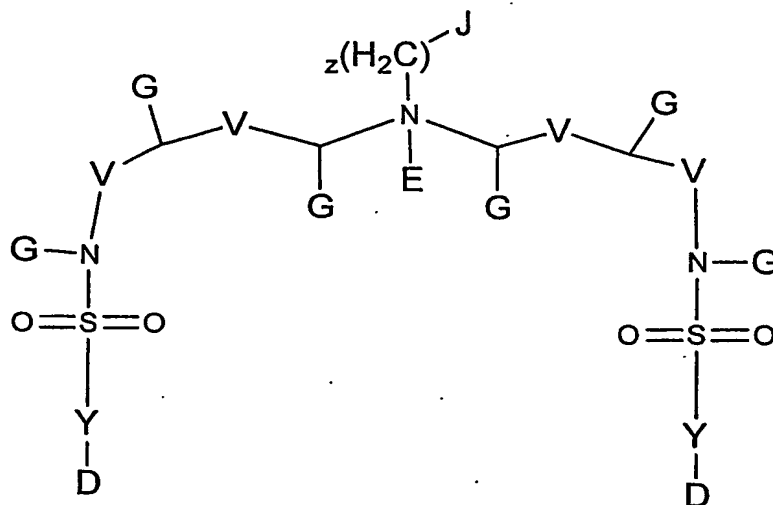
wherein V groups may be the same or different and are selected among null or -$(CH_2)_k$- ; k being an integer of 1-4;

J is selected among a hydrogen, -NH_2, -$N^+(CH_3)_3$, -$N^+[(CH_2)_mCH_3]_3$, -OH and -Q;

z is an integer of 1-8;

Y, D, E, G and Q have the same meaning as above;

and wherein the net charge of the molecule is either neutral or negative at pH of about 7; including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula II.

5   In a more specific embodiment the compounds of the present invention have the following formula III:

(III)
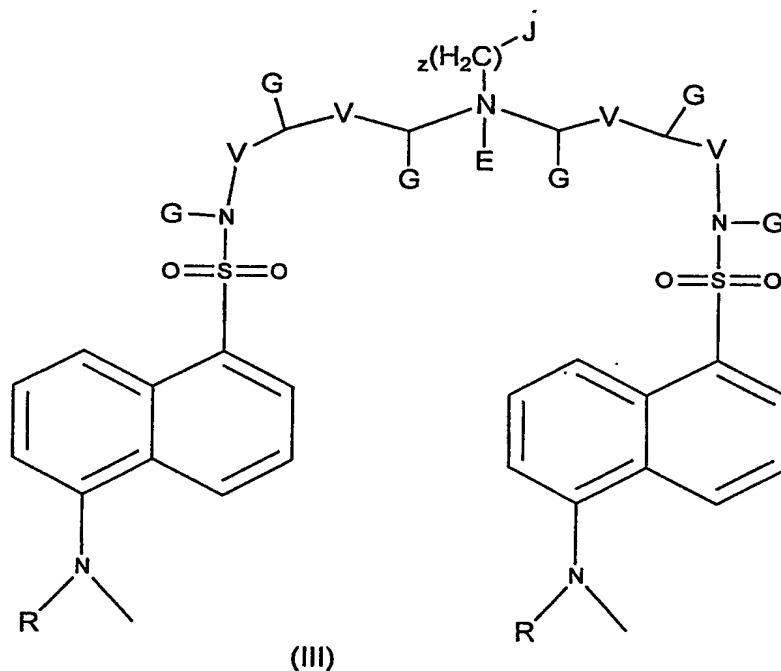
10   wherein G groups may be the same or different and are selected independently among hydrogen, $-(CH2)_m(COOH)$ and COOH wherein m is an integer between 1-3, R is a C_1-C_4 alkyl, z is an integer of 2-8 and V groups, J and E have the same meaning as above;

including pharmaceutically acceptable salts, hydrates and metal chelates of

15   the compound of the formula III.

In yet another preferred embodiment the compounds of the present invention have the following formula IV:
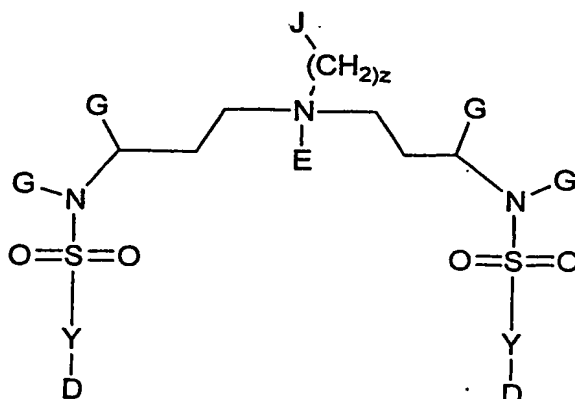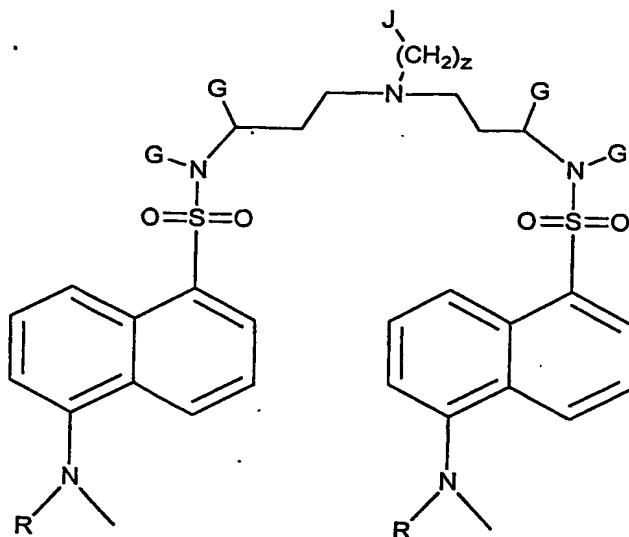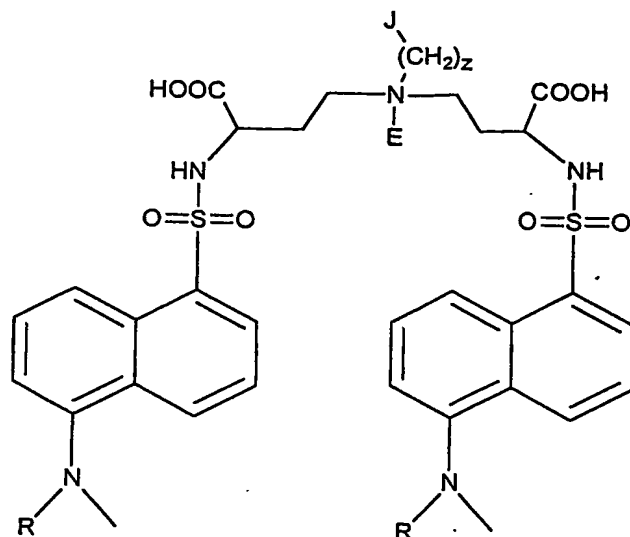
$$\text{(IV)}$$

wherein z is an intger of 2-8 and G, J, Y, D and E have the same meaning as above;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula IV.

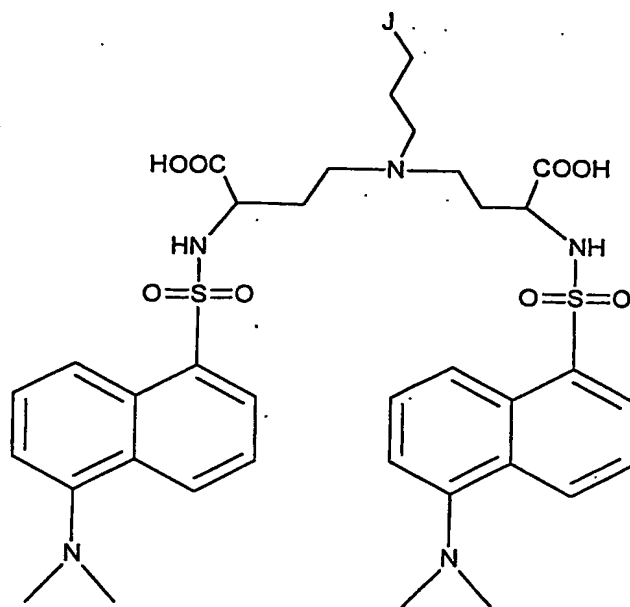In a even more preferred embodiment, the compounds of the invention have the following formula V:

$$\text{(V)}$$

wherein R, J, G and z have the same meaning as above; including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula V.

In another more preferred embodiment, the compounds of the invention have the following formula VI:

(VI)

wherein J and z have the same meaning as above, R is a C_1-C_4 alkyl and E is selected among null and a methyl group;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula VI.

Advantageously, the compounds of the invention have the following formula VII
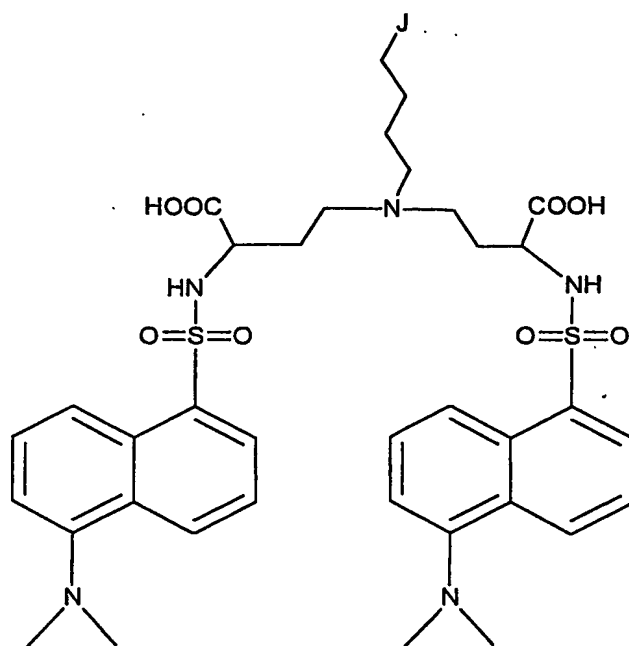
(VII)

wherein J is selected among hydrogen, –OH, –^{18}F and -Q; wherein Q is an N$_2$S$_2$ chelator;

including pharmaceutically acceptable salts and hydrates of the compound of the formula VII.

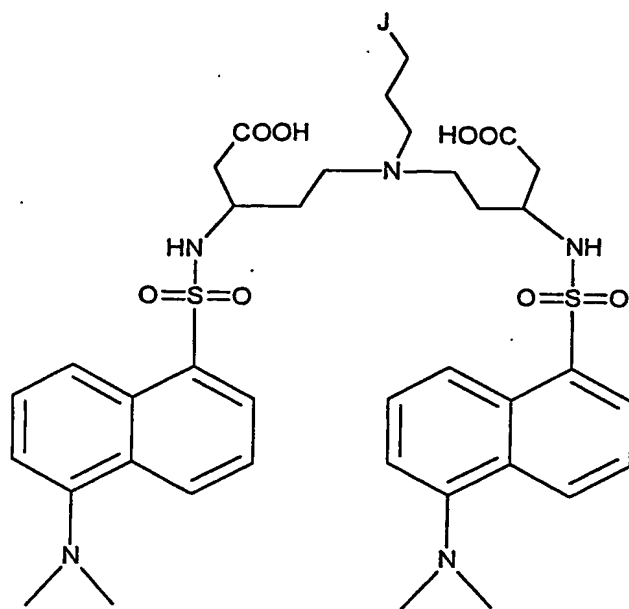In another advantageous embodiment the compounds of the invention have the following formula VIII

(VIII)

wherein J is selected among hydrogen, –OH, –^{18}F and -Q; wherein Q is an N$_2$S$_2$ chelator;

including pharmaceutically acceptable salts and hydrates of the compound of the formula VIII.

In the case that J is -OH the compound is designated NST912. In the case that J is ^{18}F the compound is designated NST913.

In another advantageous embodiment the compounds of the invention have the following formula IX:
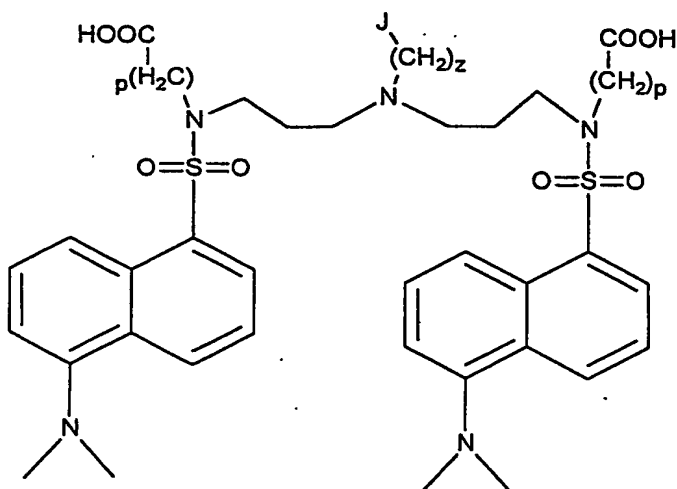
(IX)

wherein J is selected among hydrogen, –OH and ^{18}F and Q wherein Q is an N$_2$S$_2$ chelator;

including pharmaceutically acceptable salts and hydrates of the compound of the formula IX.

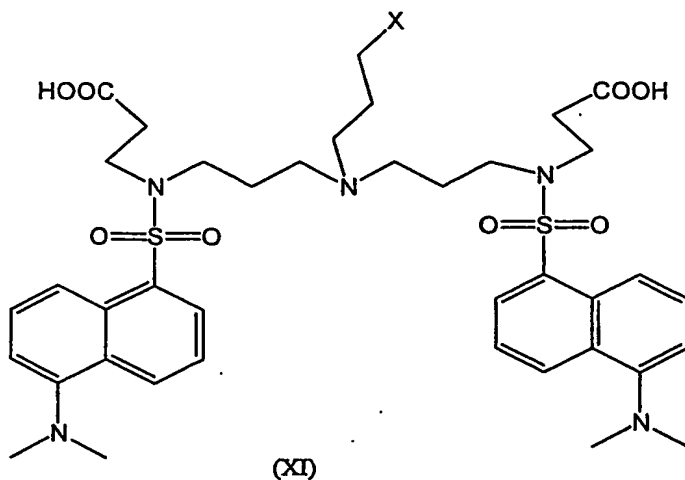In another preferred embodiment the compounds of the invention have the following formula X:

(X)

wherein p is an integer between 1-3, z is an integer between 2-6 and J is selected among –OH, ^{18}F and Q wherein Q is an N_2S_2 chelator;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula X.

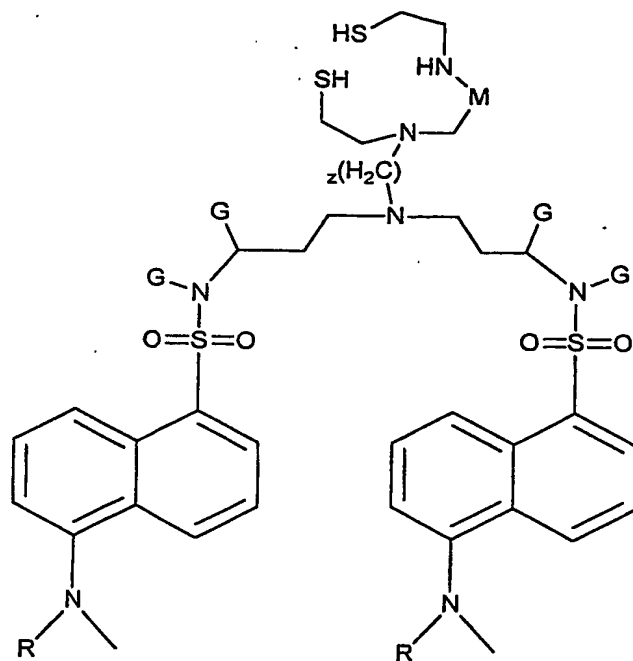Advantageously, the compound of the invention has the following formula XI:

(XI)

wherein X is selected among –OH, -^{18}F, -CH_3 and Q wherein Q is an N_2S_2 chelator;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula XI.

In yet another preferred embodiment the compounds of the invention have the following formula XII:
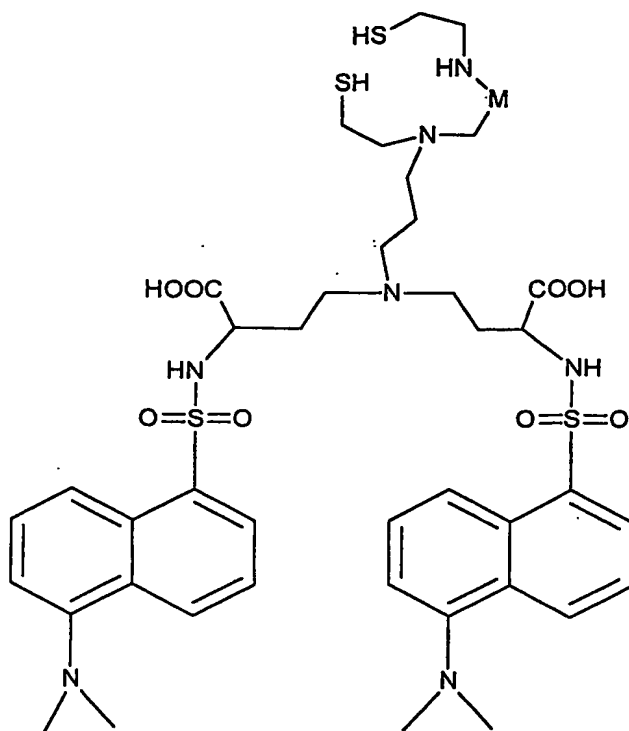

(XII)

wherein z is an integer of 2-6, R is a C_1-C_4 alkyl, and M is CH_2 or $C=O$ and G is as defined above;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula XII. Preferably, said metal chelates are Technetium or Rhenium or oxo-rhenium or oxo-technetium chelates.

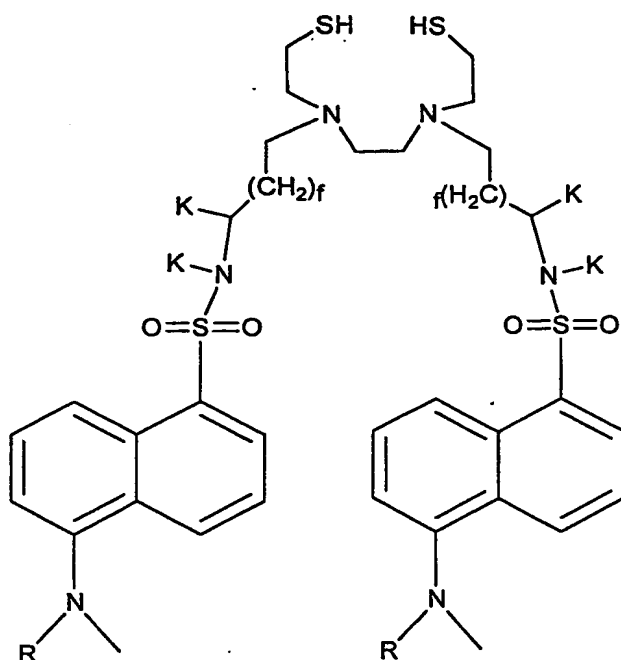Advantageously, the compound of the invention has the following formula XIII:

(XIII)

wherein M is as defined above;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula XIII. Preferably, said metal chelates are Technetium or Rhenium or oxo-rhenium or oxo-technetium chelates.

In another specific embodiment, the compounds of the invention have the following formula XIV:

(XIV)

wherein K is $-(CH_2)_p$-COOH, wherin p is as defined above and f is an integer between 0-4 and R is a C_1-C_4 alkyl;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula XIV. Preferably, said metal chelates are Technetium or Rhenium or oxo-rhenium or oxo-technetium chelates.

Advantageously, the compound of the invention has the following formula XV, and is designated NST 904:
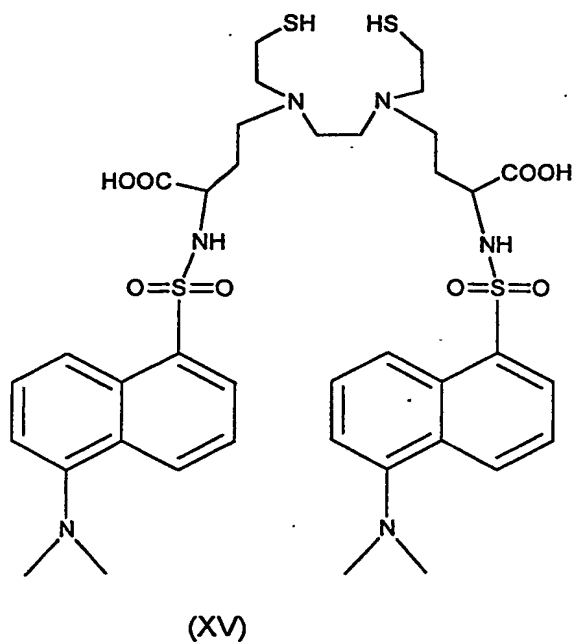
(XV)

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula XV. Preferably, said metal chelates are Technetium or Rhenium or oxo-rhenium or oxo-technetium chelates.

One desired property of the compounds of the present invention, is the selective binding of said compounds to membranes of cells, undergoing perturbation of their normal membrane organization (PNOM), with potential subsequent entry into and accumulation within said cells; while essentially binding / accumulating substantially less within cells maintaining their normal membrane organization. This property may be useful for the detection of cells or cell-derived particles, which contain **PNOM** membranes (PM), said cells being designated *"PM cells"*, and said detection being designated the *"detection aspect"* of the invention. The term PNOM for the purpose of the present invention refers to a cell membrane featuring at least one of the following:

(i) Scrambling of membrane phospholipids, with reduction of normal asymmetry of distribution of phospholipids between the inner and outer leaflets of the cell membrane;

(ii) Exposure of aminophospholipids on the outer cell surface (mainly exposure of phosphatidylserine and phosphatidylethanolamine);

(iii) Impairment of packing of membrane constituents;

(iv) Impairment of normal distribution of lipids within each membrane leaflet, such as formation of lateral domains, being either enriched or poor in a specific lipid membrane constituent, e.g., phosphatidylserine or cholesterol, respectively.

Therefore, the compounds of the invention may be used for the diagnosis of physiological disorders in which cells undergo PNOM as will be explained herein below.

Thus, according to another of its aspects, the present invention provides a novel diagnostic agent, comprising a compound of formula I as defined above, and a metal chelated to said compound through a Q moiety thereof, wherein Q is a metal chelator, said metal being the source of a signal detectable by one or more imaging techniques [e.g., radio-isotope scan, magnetic resonance imaging (MRI)].

According to another aspect, the diagnostic agent is a compound of the Formula I as defined above, wherein Q is a radioisotope of a material other than a metal, said radioisotope being the source of a signal detectable by imaging technique. Optionally, the diagnostic agent is a compound of the Formula I as defined above, having fluorescent properties that may be detected by fluorescence techniques (e.g. a fluorescent microscope).

In another aspect thereof, the present invention provides a diagnostic composition comprising an active component that is a compound of the invention of the formula I, that have detectable properties of its own or is capable of chelating a detectable label such as a metal, together with a biologically acceptable carrier, for the detection of PM cells, in a sample of biological cells, *in vitro, ex vivo, in vivo* or for clinical imaging. The active compound of the present invention is

capable of selectively binding / entering PM cells present in the assayed sample. Subsequently, said binding may be identified by any means known in the art.

According to another aspect, the present invention provides a diagnostic kit for the administration of a diagnostic composition or a diagnostic agent to a subject in order to diagnose a physiological disorder. Such diagnostic kit comprises one or more vials containing a sterile formulation comprised of a predetermined amount of a diagnostic composition or diagnostic agent of the invention and optionally other components, such as stabilization aids, solubilization aids or bacteriostats. The one or more vials that contain all or part of the formulation can independently be in the form of a sterile solution or a lyophilized solid.

In a preferred embodiment, the diagnostic composition is a diagnostic radiocomposition, for radioimaging by standard radioimaging techniques, such as single photon emission computed tomography (SPECT), the metal being a radioisotope of the following metal atoms: Tc, In, Cu, Ga, Xe, Tl and Re, preferably Tc, Tc=O, Re and Re=O; or the covalently linked radiolabel is selected from: ^{123}I and ^{131}I . For example, a preferred embodiment is a compound of the invention, radiolabeled with ^{99m}Tc=O, for detection by SPECT.

In another preferred embodiment, the diagnostic composition is a diagnostic radiocomposition for Positron Emission Tomography (PET) scan, comprising a covalently linked radiolabel (the Q moiety), selected from the group ^{18}F, ^{15}O, ^{18}O, ^{11}C, ^{13}C, ^{124}I, ^{13}N and ^{75}Br.

In yet another preferred embodiment, the diagnostic composition is a MRI contrast composition, the metal being a paramagnetic metal ion selected from: Gd(III), Fe(III) or Mn(II).

In yet another preferred embodiment, the diagnostic composition is an X-ray or computerized tomography (CT) contrast composition, comprising a contrast agent such as Ba, Cs, Re, Rh, Ag, Ir or iodine.

The term *"disease characterized by PM"* refers to a disease of which one of its manifestations is PM cells, occurring in tissues inflicted by the disease. This is not meant to indicate that this perturbation of normal membrane structure of these

cells is necessarily the cause, or the sole effect of the disease, but rather that it is one of its manifestations.

The compounds and the diagnostic agents of the invention can be used for a detection of the following conditions:

5   1)   apoptotic cells and apoptotic bodies;

2)   damaged cells and cells undergoing non-apoptotic modes of cell death;

3)   activated platelets;

4)   activated inflammatory cells, such as activated white blood cells or tissue macrophages.

10   Therefore, the compounds and the agents of the invention can be useful in the diagnosis of a wide variety of biological conditions, in which the above cells and cell-derived particles have a role. These include physiological conditions such as tissue development or aging, and various pathological conditions.

According to the *detection aspect* of the invention, the compounds of the

15   invention may be used for the diagnosis of medical disorders in which cells undergo PNOM, monitoring the progression of said medical disorders or monitoring the effects of treatments administered to patients suffering from said diseases. Examples of such medical disorders are as follows:·

Diseases which are characterized by occurrence of excessive apoptosis,

20   such as degenerative disorders, neurodegenerative disorders (e.g., Parkinson's disease, Alzheimer's disease, Huntington chorea), AIDS, myelodysplastic syndromes, ischemic or toxic insults, graft cell loss during transplant rejection; tumors, and especially highly malignant / aggressive tumors, are also often characterized by enhanced apoptosis, in addition to the excessive tissue

25   proliferation.

Diseases manifested by excessive blood clotting, wherein PNOM occur during platelet activation, and / or during activation of or damage to other cellular elements (e.g., endothelial cells). These diseases include, among others, arterial or venous thrombosis, thrombo-embolism, e.g., myocardial infarction, cerebral stroke,

30   deep vein thrombosis, disseminated intravascular coagulation (DIC), thrombotic

thrombocytopenic purpura (TTP), sickle cell diseases, thalassemia, antiphospholipid antibody syndrome, systemic lupus erythematosus.

Inflammatory disorders, and / or diseases associated with immune–mediated etiology or pathogenesis, such as auto-immune disorders such as
5   antiphospholipid antibody syndrome, systemic lupus erythematosus, connective tissue disorders such as rheumatoid arthritis, scleroderma; thyroiditis; dermatological disorders such as pemphigus or erythema nodosum; autoimmune hematological disorders; autoimmune neurological disorders such as myasthenia gravis; multiple sclerosis; inflammatory bowel disorders such as ulcerative colitis;
10   vasculitis.

Atherosclerotic plaques, and especially plaques that are unstable, vulnerable and prone to rupture, are also characterized by PM cells, comprising apoptotic macrophages, apoptotic smooth muscle cells, apoptotic endothelial cells, activated platelets and activated inflammatory cells. In addition, the compounds of the
15   present invention are capable of binding to the extracellular lipid, characterizing said atherosclerotic plaques, and thus allow imaging of said lipid core of the atherosclerotic plaque.

The detection of these pathological conditions, disorders or diseases via detection of the PM cells may be an aim by itself, simply for diagnosis of the
20   presence of a disease condition in a specific individual.

Said detection may also be carried out in a person already known to have the disease for the purpose of evaluating the disease severity and / or in order to monitor response to various therapeutic modalities. An example for such monitoring is evaluation of response to anticancer therapy. Since most anti-tumor
25   treatments, chemotherapy or radiotherapy exert their effect by induction of cell death, and particularly apoptosis, detection by the agents of the invention of therapy-induced apoptosis of tumor cells may substantially assist in the proper evaluation of the efficacy of said treatments.

Moreover, said detection may be used to monitor adverse effects of
30   anti-cancer treatments. A large part of such adverse effects are due to untoward

treatment-induced apoptosis of normal, yet sensitive cells, such as various types of epithelial cells or cells of the bone marrow hematopoietic system. Detection by the compounds of the invention of such apoptosis may allow early detection of this untoward tissue damage and better optimization of the treatment protocol.

5   In addition, said detection may aim at characterization of intrinsic apoptotic load within a tumor, characterization of the level of aggressiveness of a tumor, and detection of metastases.

Similarly, the compounds of the current invention may be useful in monitoring graft survival after organ transplantation, since apoptosis, potentially

10   detectable by the compounds of the invention, plays a major role in cell loss during graft rejection. Early diagnosis of such rejection is of major clinical importance, and is currently being achieved by recurrent invasive and potentially dangerous tissue biopsies. The method of the invention may be useful as a non-invasive method for detecting cell death within the graft, and its imaging.

15   In addition, said detection may be useful for monitoring response to cyto-protective treatments, administered to inhibit cell death in disorders such as degenerative disorders or various ischemic and toxic insults. Thus the compounds and method of the present invention may aid in the screening and development of drugs which are capable of inhibiting cell loss in various diseases (for example

20   those recited above) by enabling a measure of evaluation of cell death.

Said detection may be also useful for detection of atherosclerotic plaques, since the destabilization of such plaques, rendering them vulnerable, prone to rupture, thrombosis and embolization, is characterized by participation of several elements, of which have in common perturbed membranes: (i). apoptotic cells: the

25   unstable plaque is characterized by apoptotic macrophages, apoptotic smooth muscle cells, and apoptotic endothelial cells (ii). activated platelets (iii). activated inflammatory cells. A lipid core, comprising of extracellular accumulation of lipids is also one of the hallmarks of atheroscleorotic plaques undergoing destabilization (Kockx M.M., et al., 2000; Stary, H.C., et al., 1995). The compounds of the

invention may be useful, due to their hydrophobic aromatic component, also for the binding to and identification of such lipid core of the atherosclerotic plaque.

The detection may also take place for basic research purposes in the study of apoptosis in tissue culture and animal models, and may help in determining the role of apoptosis not only is disease states, but also in normal development and homeostasis of various tissues, such as in the development of the central nervous system during embryogenesis, as well as during situations such as normal aging.

In accordance with this approach, the present invention further concerns a method for the detection of PM cells , the method comprising:

(i) contacting the cell sample with a diagnostic agent of the invention under conditions enabling binding / accumulation of said agent in cells;

(ii) detecting compound in said cells; the presence of a significant amount of compound in cells indicating the presence of PNOM in said cells.

The method of the present invention may be used for the diagnosis of a disease characterized by the occurrence of PNOM, for example, any one of the diseases indicated above.

The method of the present invention may also be used for monitoring the effects of various therapeutic modalities for said diseases or medical conditions, or alternatively for basic science research purposes as explained above.

The composition of the invention may be administered by any of the known routes, *inter alia*, oral, intravenous, intraperitoneal, intramuscular, subcutaneous, sublingual, intragastric, intraocular, intranasal or topical administration. The carrier should be selected in accordance with the desired mode of administration, and include any known components, e.g. solvents; emulgators, excipients, talc; flavors; colors, etc. The pharmaceutical composition may also comprise, if desired, also other pharmaceutically-active compounds which are used to treat disease, eliminate side effects or augment the activity of the active component.

The present invention further provides a novel method for the detection of physiological disorders characterized by the presence of PM cells, and / or diseases

in which PM cells have an etiological or a pathogenetic role, such method comprising:

(1) administering a diagnostic composition of the present invention to a patient; and

(2) imaging of the patient using an appropriate imaging technique, known to those of art.

In a preferred embodiment, the present invention provides a novel method for the detection of physiological disorders characterized by the presence of PM cells, and / or diseases in which PM cells have an etiological or a pathogenetic role, such method comprising:

(1) administering a radiocomposition of the invention to a patient; and

(2) imaging of the patient using radioimaging techniques known to those of the art, such as single photon emission tomography (SPECT) in the case of a radiocomposition comprising ^{99m}Tc, or positron emission tomography (PET) in the case of a radiocomposition comprising ^{18}F.

In another preferred embodiment, the present invention provides a novel method for the detection of physiological disorders characterized by the presence of PM cells, and / or diseases in which PM cells have an etiological or a pathogenetic role, such method comprising:

(1) administering a MRI contrast composition of the invention to a patient; and

(2) imaging the patient using magnetic resonance imaging techniques, known to those of the art.

In yet another preferred embodiment, the present invention provides a novel method for the detection of physiological disorders characterized by the presence of PM cells, and / or diseases in which PM cells have an etiological or a pathogenetic role, such method comprising:

(1)   administering a X-ray contrast composition of the present invention to a patient; and

(2)   imaging the patient using X-ray or computed tomography (CT) techniques, known to those of the art.

5   In yet another preferred embodiment, the present invention provides a novel method for the detection of physiological disorders characterized by the presence of PM cells, and / or diseases in which PM cells have an etiological or a pathogenetic role, such method comprising:

(1)   administering a composition of the present invention to a patient;
10   wherein the diagnostic agent is a fluorescence-emitting moiety; and

(2)   imaging the patient using fluorescence techniques known to those of the art (e.g. a fluorescent microscope).

15

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried-out in
20   practice, a preferred embodiment will now be described, in which detection of binding / entering of compounds of the present invention to cells undergoing PNOM due to apoptosis or cell activation was evaluated. Selective binding of NST912 is presented. Binding was measured by monitoring of the intensity of the intrinsic fluorescence of the compounds by using fluorescent microscopy detection
25 analysis. Said preferred embodiment will now be described by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 shows flow-cytometric analysis demonstrating the selective binding of NST912 to Jurkat cells in the early stages of apoptosis induced by anti-Fas
30 antibody;

Fig. 2 shows detection of apoptosis of small intestine epithelial cells, induced in mice by systemic administration of chemotherapy; said detection being performed by NST912 *in vivo*.

5

DETAILED EXPLANATION OF FIGURES:

Fig. 1: Flow-cytometric analysis demonstrating the selective binding of NST912 to apoptotic Jurkat cells:

10

Jurkat cells were induced to undergo apoptosis by exposure to 0.1 μg/ml anti-Fas antibody for 2 hours. Non-treated cells served as control. The cells were then incubated with NST912 (50 μM) for 30 min. and further subjected to FACS analysis, using a Becton-Dickinson cell sorter and a CellQuest software. Excitation

15   was at 356nm and emission was at 530nm.

Control, non-treated cells exhibit a major peak of low fluorescence (Fig. 1 blue line). The smaller peak of higher fluorescence value represents the population of cells in late apoptosis, naturally-occurring within all cultures. Induction of apoptosis was associated with a shift of the cell population and emergence of a

20   novel peak at higher fluorescence (Fig.1; red line). It reflects selective binding and accumulation of NST912 by these cells in early apoptosis. The X axis represents the fluorescence in 530 nm, and the Y axis represents the number of cells.

Fig. 2: Detection of chemotherapy-induced apoptosis of small intestine
25   **epithelial cells *in vivo* by NST912:**

Balb/c mice were treated with a single dose of chemotherapy [Taxol (27 mg/kg) + cyclophosphamide (300 mg/kg)]. Twenty-four hours later, the animals were injected intravenously with NST912. Animals were sacrificed two hours later, and the small intestine tissue was removed and subjected to fluorescent

histopathological analysis. Numerous cells within the intestinal crypta undergoing apoptosis demonstrated selective uptake and accumulation of the compound NST912. No such staining was observed in the small intestine of control animals, not receiving chemotherapy.

EXAMPLES:

Example 1: Synthesis of thiol-protected NST904 (Scheme 1):

4-Methoxybenzyl chloride (15.6g) was reacted with 2-aminoethanethiol (7.7g) in methanol and sodium methoxide, to afford of 2-(4-methoxybezyl-sulfanyl)-ethylamine (1) (18.8g). One half of this material was taken in dichloromethane (cooling bath 0°C). Chloroacetyl chloride in the same solvent, was added slowly with stirring, followed by an equivalent of triethyl amine to produce 2-chloro N-[2-(4-methoxybenzyl-sulfanyl)-ethyl]acetamide (2) in a 94% yield. Compound 2 was reacted with an equivalent amount of 1 in refluxing acetonitrile for 6 hrs to provide N-[-2-(4-methoxy-benzylsulfanyl)-ethyl]-2-[2-(4-methoxy-benzylsulfanyl)-ethylamino]-acetamide (3) (40.4g) . Compound 3 was reduced with lithium aluminium hydride (LAH) to yield 4 in 85%.

To a cold solution of anhydrous methanol was added dropwise 10 equivalents of thionyl chloride so as to maintain the internal temperature below 10 °C. Reaction mixture was further stirred for 1 hour and treated with10 g (L)-4-bromo-2-aminobutyric (5). The reaction was slowly warmed to room temperature (RT) over 1 hour and stirred at 40 °C overnight. Volatiles were removed under reduced pressure to dryness, and residue triturated with dichloromethane (DCM) to afford an off white solid (12 g) (6). That solid was dissolved in tetrahydrofuran (THF), treated with 3 eq. diisopropyl ethyl amine (DIPEA) followed by dibutyl oxycarbonyl anhydride (Boc$_2$O). The reaction was stirred at RT for 6 hours. The reaction mixture was diluted with ethyl acetate (100 mL) and the combined organics washed with saturated ammonium chloride

(100 mL), brine (100 mL) and dried over sodium sulfate and filtered. The filtrate was concentrated to dryness to afford 11 g of butyloxycarbonyl-2-amino-4-bromobutyric acid methyl ester (7) as a thick oil which slowly crystallized.

Compound 7 was taken up in acetone (10 volumes) and treated with 5 equivalents of sodium iodide. The resulting yellowish solution was refluxed overnight. The reaction was then concentrated to dryness, the residue dissolved in water (150 mL) and extracted with DCM (150 mL). The organic layer was collected, washed with brine, dried and concentrated to dryness to provide 8 g of 8 as a pale oil, purity of 94%(HPLC).

1.3 g of diamine 4 were treated with 2.4 g of iodide 8 in acetonitrile with DIPEA (3 eq) as the base. The reaction was then stirred at 70 °C for 5 hours followed by further stirring at the same temperature overnight. After flash chromatography, Butyl oxycarbonyl-2-amino-4-{(2-{(butyl oxycarbonyl 3-amino-3-methoxycarbonyl-propyl)-[2-(4-methoxy-benzylsulfanyl)-ethyl]-amino}-ethyl)-[2-(4-methoxy-benzylsulfanyl)-ethyl]-amino}-butyric acid methyl ester (9) was obtained (40% yield).

Treatment of 9 (0.9 g) with 10 equivalents of 4M HCl/dioxane solution overnight afforded the dihydrochloride salt 10 in excellent purity and good yield. 5-Dimethylamino-1-naphthalenesulfonyl (dansyl) linkage was achieved by reacting 10 with dansyl chloride and DIPEA to provide 0.9 g of 11 with a purity of >98% after column chromatography. Hydrolysis of the esters was carried in a mixture of dioxane/water (2:1) overnight to provide >95% conversion of the di-ester to 2-Amino-4-{(2-{(3-amino-3-carboxy-propyl)-[2-(4-methoxy-benzylsulfanyl)-ethyl]-amino}-ethyl)-[2-(4-methoxy-benzylsulfanyl)-ethyl]-amino}-butyric acid (thiol-protected NST904).

At this point the reaction mixture was of a milky suspension and the mixture was diluted with water (10 mL) and the organic solvent removed *in vacuo*. The milky aqueous layer was then covered with ethyl acetate and acidified

to pH 3.0 with 2.0 M HCl. The organic layer was collected, dried and concentrated to dryness to afford an off-white solid of **thiol-protected NST904** (1.3 g) which was practically pure. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.87 (d, J = 8.87 Hz, 2H), 8.70 (d, J = 8.56 Hz, 2 H), 8.40 (d, J =7.31Hz, 2 H), 7.98 (d, J = 7.63 Hz, 2 H), 7.81 (m, 4 H), 7.30 (d, J = 8.58 Hz, 2 H) 6.87 (d, J = 8.62 Hz, 2 H), 4.05 (dd, J = 3.91 Hz, J = 4.02 Hz, 2 H), 3.58 (bs, 4H), 3.3(, s, 12 H), 3.29 (m, 10H), 2.77 (m, 4H), 2.23 (m, 2H), 2.02 (m, 2H). Melting range (DSC) ; MS(ESI) calcd 1088, found 1089 (M+ 1). IR (KBr), 3442.86, 2950.0, 1729.97, 1612.46, 1516.32, 1462.91, 1331.16, 1252.82, 1145.99 cm^{-1}. HPLC Hypersil BDS, C18 4.5 x 150 mm, 5μ. Solvent A: H$_2$0/0.1%TFA, solvent B Acetonitrile/0.1% TFA; gradient of 30% B to 100% B over 17.0 min, flow rate 1.0 mL/min, detector @ 254 nm, Rt 10.318 (94.0%).

Scheme 1

5

10
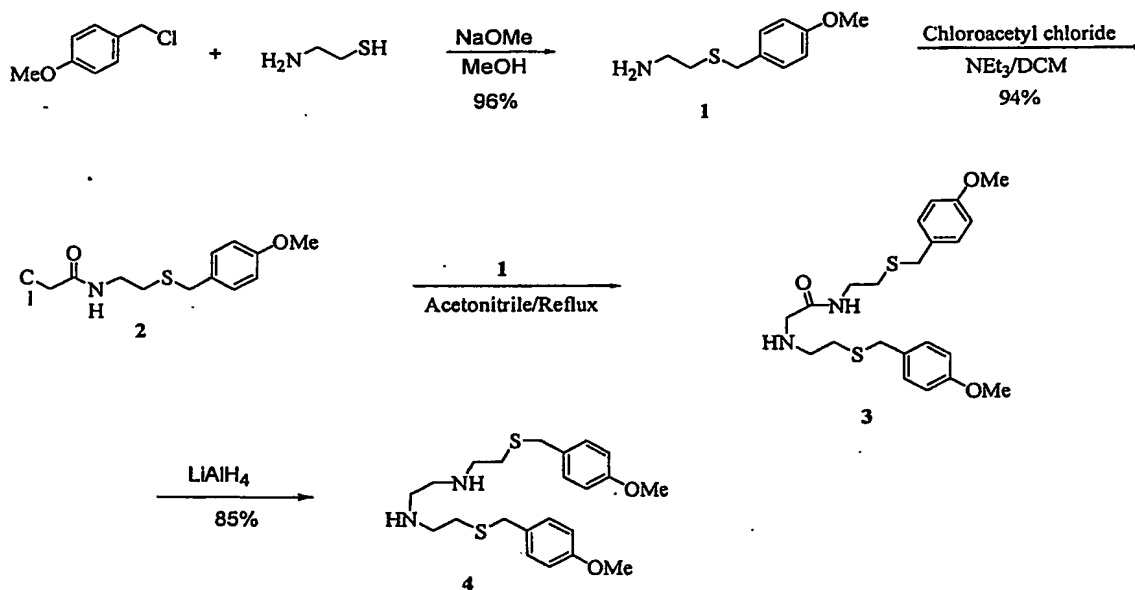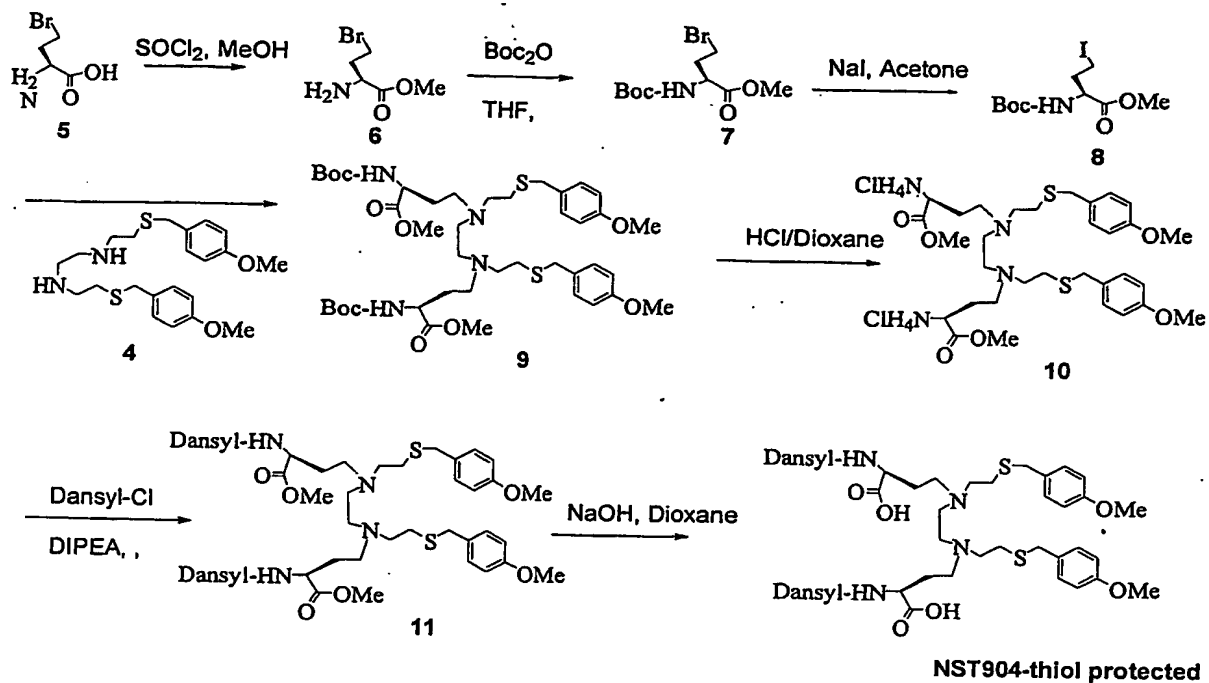
Example 2: Synthesis of NST912 (Scheme 2):

Compound **8** (4.4 g) was reacted with 4-amino-1-butanol to afford 1.0 g of 4-[(4-Hydroxy-butyl)-(3-methoxycarbonyl-3-*t* butyloxycarbonylamino-propyl)-amino]-2-*t* butyloxycaonylamino-butyric acid methyl ester **12**. The Boc groups were removed in the usual manner (HCl/Dioxane) yielding **13**, and the amine functions were dansylated as specified in Example 1 to yield **14**. In order to remove the ester groups 8 equivalents of KOH were added to **14** and the reaction was stirred for 39 hours yielding NST912 in 81% yield -^{1}H NMR (300 MHz, CDCl₃) δ 8.53 (d, *J* = 8.4 Hz, 2H), 8.35 (d, *J* = 8.4 Hz, 2H), 8.23 (d, *J* = 7.2 Hz, 2H), 7.59 (t, *J* = 8.1 Hz, 2H), 7.56 (t, *J* = 7.5, 2H), 7.24 (d, *J* = 7.5 Hz, 2H), 3.80-3.70 (m, 2H), 3.55-3.45 (m, 2H), 2.85 (s, 12H), 2.83-2.60 (m, 5H), 2.05-1.80 (m, 5 H), 1.55-1.35 (m, 4H). MS for $C_{36}H_{47}N_5O_9S_2$ calcd 757, found 758 (M + 1).
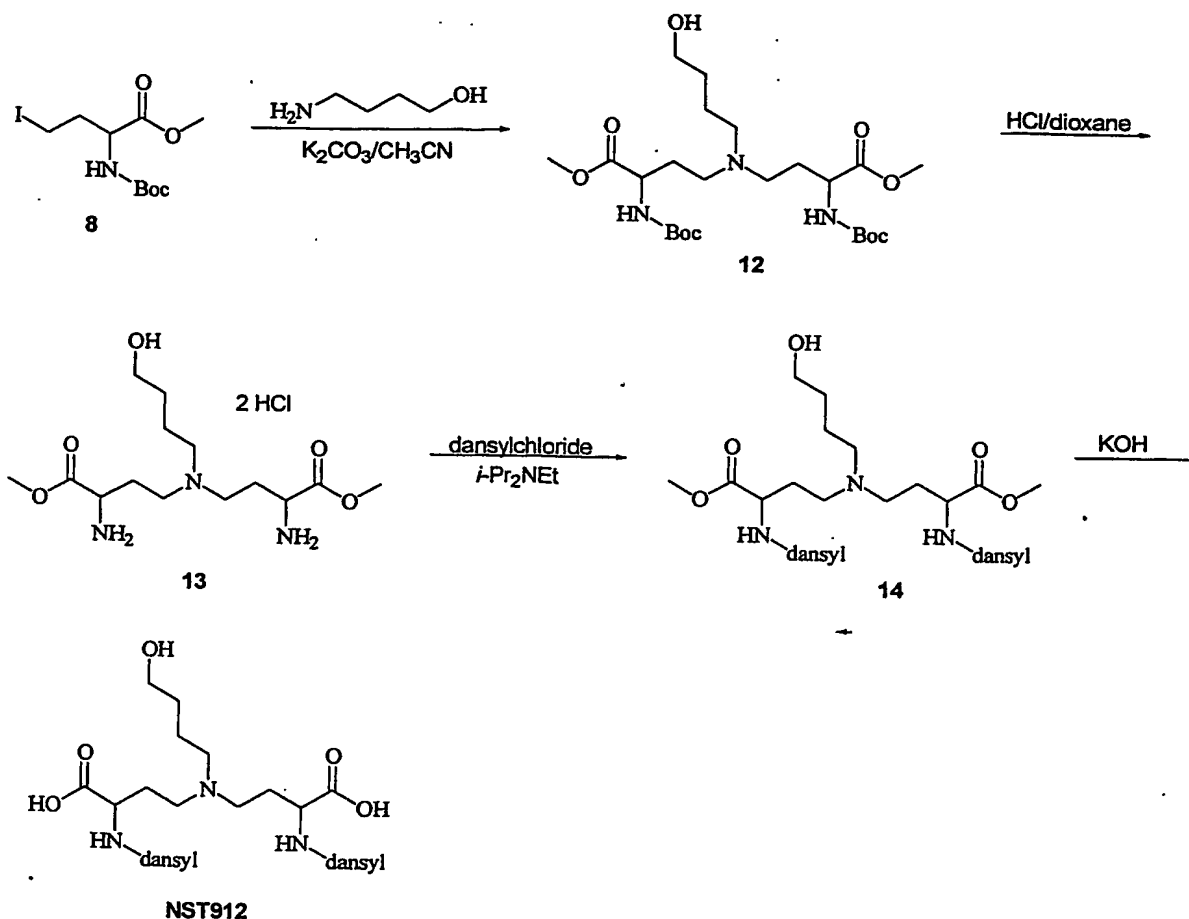
Example 3: Selective binding of NST912 to apoptotic cells; flow-cytometric analysis:

Human adult T cell leukemia (Jurkat cells) were grown in RPMI 1640 medium supplemented with 2mM of L-glutamine; 100 units/ml of penicillin; 100 µg/ml of streptomycin; 12.5 units/ml of nystatin; 1 mM sodium pyruvate, 1mM HEPES and 10% fetal calf serum (FCS). Cells were grown in suspension in vertical flasks and seeded at a density of 5×10^6 in 10 ml medium. To induce apoptosis, 1×10^6 cells/ml were treated with IgM anti-Fas antibody at a concentration of 0.1 µg/ml for 120-180 min. Cells were harvested followed by centrifugation at 1600 RPM for 5 min.

The cells were then subjected to analysis by flow cytometry (FACS) using Beckton-Dickinson cell sorter and CellQuest software. Excitation was at 356nm and emission was measured at 530nm. As shown in the FACS histogram in Fig. 1, non-treated cells manifest low levels of fluorescence values upon addition of NST912 (blue line). Apoptotic cells shifted to a distinct peak of higher fluorescence levels (red line), manifesting selective uptake of NST912 by theses cells. NST912 can therefore act, through its detection of PNOM, as a potent agent to mark and distinguish between apoptotic and non-apoptotic cells.

Example 4: Detection of chemotherapy-induced apoptosis of mouse small intestine epithelial cells *in vivo* by NST912:

Gastrointestinal damage is often observed during administration of anti-cancer therapy. In particular, the small intestine crypts manifest apoptosis of epithelial cells as an early response to chemotherapy and irradiation (Keefe, D.M.K., *et al.*, *Gut*, 47:632-637, 2000). Detection of chemotherapy-induced, small intestine epithelial apoptosis by NST912 *in vivo* was therefore examined.

Twelve-week old Balb/c mice were treated intravenously with a single dose of a combination of Taxol (27 mg/kg) and cyclophosphamide (300 mg/kg). After 24 hours, all animals were injected intravenously with of NST912 (2 mg per animal). Two hours later, animals were sacrificed, small intestine was removed and cryo-sections were prepared for fluorescent microscopy.

Strong, selective uptake of NST912 by apoptotic cells was detected in the small intestinal crypts of the chemotherapy-treated mice (Fig. 2). By contrast, no significant uptake of NST912 was observed in non-apoptotic cells in the crypts of chemotherapy-treated mice (Fig. 2), or in tissues obtained from animals not treated with chemotherapy. This exemplifies the potential usefulness of the compounds of the invention as a tool for an early and sensitive monitoring of this adverse effect of chemotherapy, allowing its detection even after a single dose of anti-cancer treatment.

CLAIMS:

1. A compound having the formula (I):
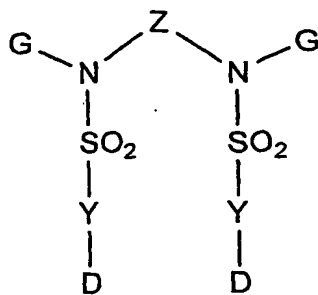
$$G\text{---}N(\text{---}Z\text{---})N\text{---}G$$

$$Y\text{---}D$$

(I)

including pharmaceutically acceptable salts, metal chelates and hydrates of the compound of formula (I); wherein

Y groups may be the same or different, each being an aromatic, optionally heterocyclic, 8-10 atom, two-ring system;

G groups may be the same or different and are selected independently among hydrogen–$(CH2)_m CH(NH_2)(COOH)$, –$(CH2)_m(COOH)$ and $(CH2)_m NH_2$; m being an integer of 0-4;

D is WR_b, wherein W is selected among null, N, O, S and C;

R represents hydrogen or a C_1-C_6 alkyl; R moieties may be either the same or different; and

b is 1, 2 or 3.

Z is selected from a group having the formula U(A)-T-U(A)- and a group having the formula -U-A-U-T-U-A-U-,

U groups may be the same or different, and are each independently selected from null or an optionally substituted C_1-C_4 alkylene, C_2-C_4 alkenylene, C_3-C_8 branched alkylene, and C_3-C_8 branched alkenylene;

A groups may be the same or different, and are selected among null, hydrogen and charged groups at pH of about 7; charge(s) of said groups being

selected among positive charge(s), negative charge(s), and zwitterion forms; wherein at least one A group is other than null and the total charge of the compound is either negative or zwitterion;

T is selected among -A-, -A(B)-, -(E)A(B)-, -Q-, -Q(B)-, and -A(B-Q)-, wherein A has the same meaning as described above;

B is selected from hydrogen or an optionally substituted C_1-C_6 alkyl, C_1-C_6 alkylene, C_2-C_6 alkenyl, C_2-C_6 alkenylene, C_3-C_8 branched alkyl, C_3-C_8 branched alkylene, C_3-C_8 branched alkenyl, C_3-C_8 branched alkenylene, aryl, heteroaryl and combinations thereof;

E is selected from null, hydrogen and a C_1-C_6 alkyl,

and

Q is selected among null, a marker for imaging, or a metal chelator; said marker for imaging being selected from the group comprising a fluorescent label, a radio-label, a marker for X-ray, a marker for MRI, a marker for PET scan, or a label capable of undergoing an enzymatic reaction that produces a detectable color;

2.   The compound of Claim 1, wherein Q is a metal chelator.

3.   The compound of Claim 1, wherein Q is selected among ^{18}F, ^{13}C and ^{124}I.

4.   The compound of Claim 3, wherein Q is covalently linked to Y or D moieties wherein Y and D are as defined in Claim 1.

5.   The compound of anyone of Claims 1-4, wherein U are each independently selected among null and an optionally substituted C_1-C_3 alkylene group; D is NR_2, wherein each R group is selected independently from hydrogen and C_1-C_4 alkyl.

6.   The compound of Claim 1 wherein A comprises acidic group(s) being selected from carboxylic, phosphoric, sulfonic and sulfuric acid;

7.   The compound of Claim 2 where Q forms metal chelates with Technetium, oxo-technetium, Gallium, Rhenium or oxo-rhenium isotopes.

8.   The compound of Claim 2, where the chelation of Q to a metal is accomplished through a combination of nitrogen, sulfur and/or oxygen atoms comprised by Q.

9. The compound of Claim 8, wherein the chelation of Q to a metal is accomplished through a combination of three nitrogen atoms and a sulfur atom, two nitrogen atoms and two sulfur atoms or a nitrogen atom and three sulfur atoms.

10. The compound of Claim 9, wherein Q is selected from diaminedithiols, monoamine-monoamide-bisthiols (MAMA), triamide-monothiols, and monoamine-diamide-monothiols.

11. The compound of Claim 1 having the following formula (II):
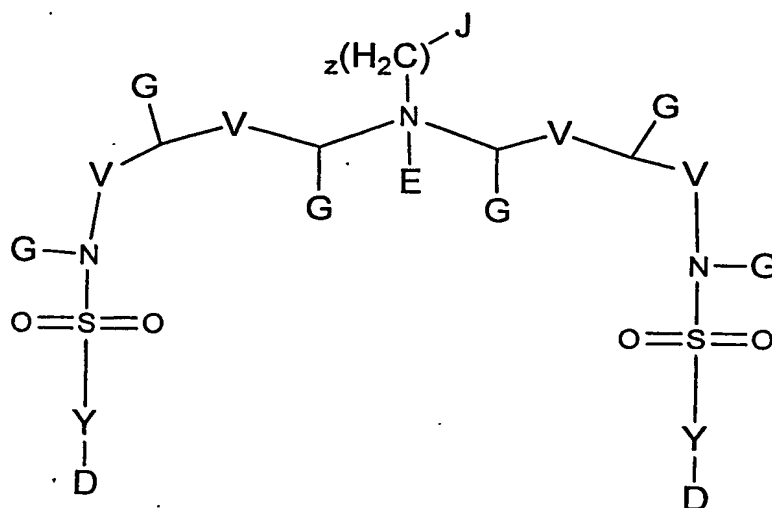
(II)

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula II;

wherein V groups may be the same or different and are selected among null or -$(CH_2)_k$-; k being an integer of 1-4;

J is selected among null, -NH_2, -$N[(CH_2)_mCH_3]_3$, -OH and Q;

z is an integer of 1-8;

Y, D, E, G and Q are as defined in Claim 1; and

the net charge of the molecule is either neutral or negative at pH of about 7.

12. The compound of Claim 11 having the following formula (III):
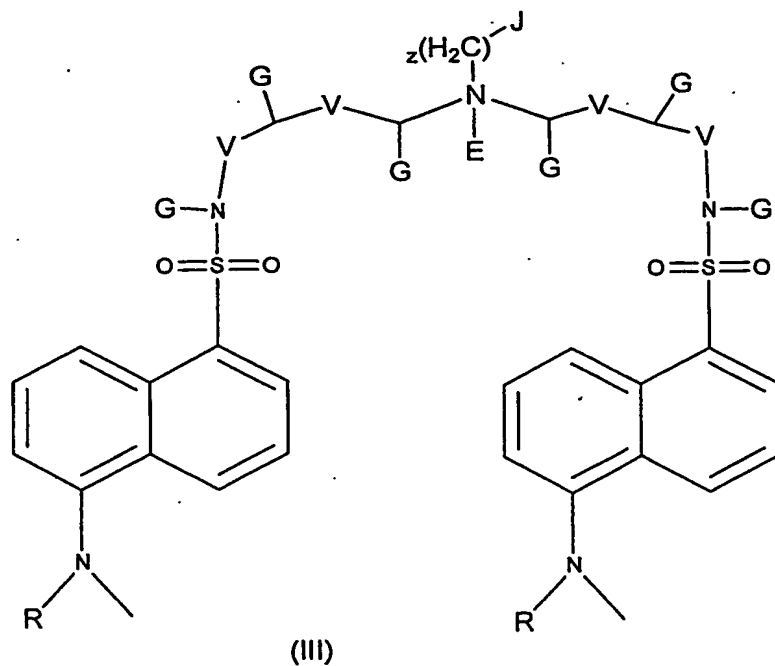
(III)

wherein G groups may be the same or different and are selected independently among hydrogen, −(CH2)$_m$(COOH) and COOH wherein m is an integer between 1-3, R is a C_1-C_4 alkyl, z is an integer of 2-8; V groups and J are as defined in Claim 11 and E is as defined in Claim 1;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula III.

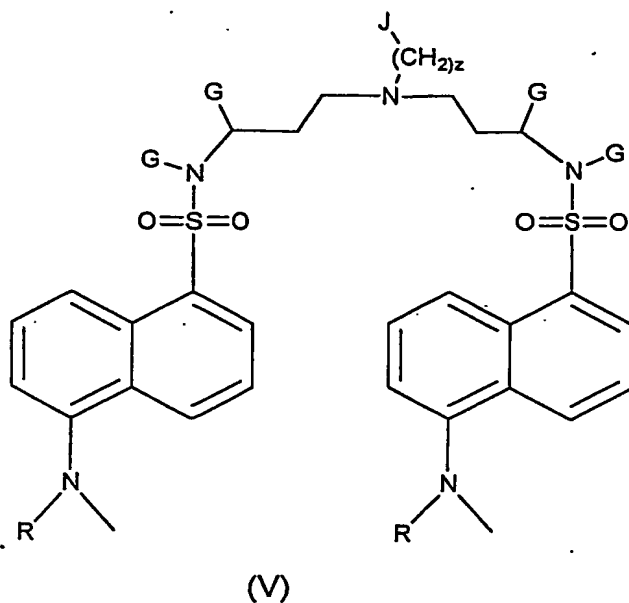13.   The compound of Claim 11 having the following formula (IV):

(IV)
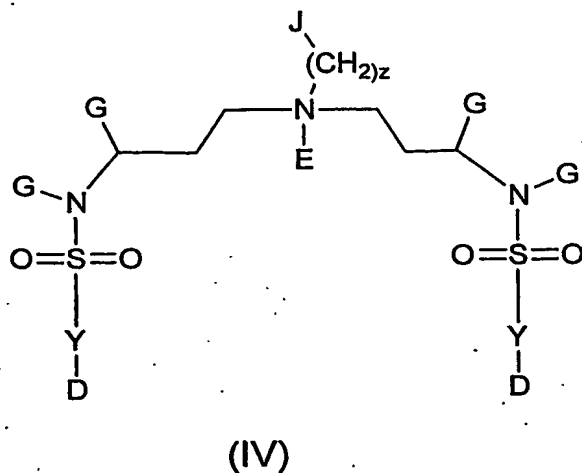
wherein Y, E, G and D are as defined in Claim 1 and J and z are as defined in Claim 12;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula IV.
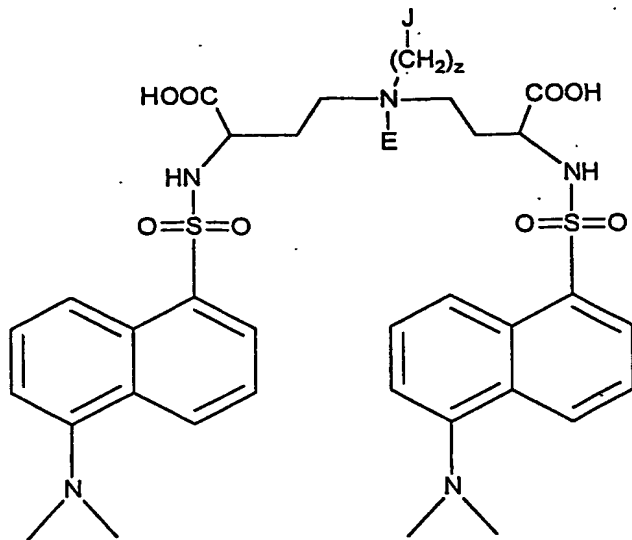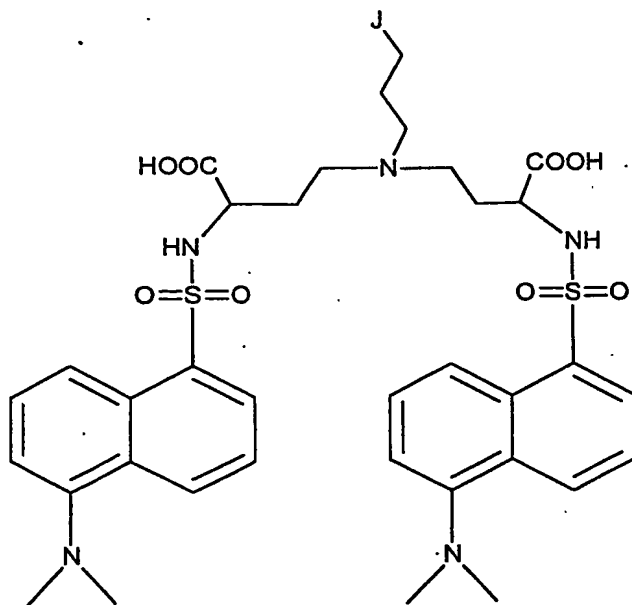
14.   The compound of Claim 13 having the following formula V:

(V)

wherein R and G are as defined in Claim 1 and J and z are as defined in Claim 12;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula (V).

15.   The compound of Claim 14 having the following formula VI:

(VI)

wherein J is selected among –OH and ^{18}F; E is selected among null and a methyl group; including pharmaceutically acceptable salts and hydrates of the compound of formula (VI).
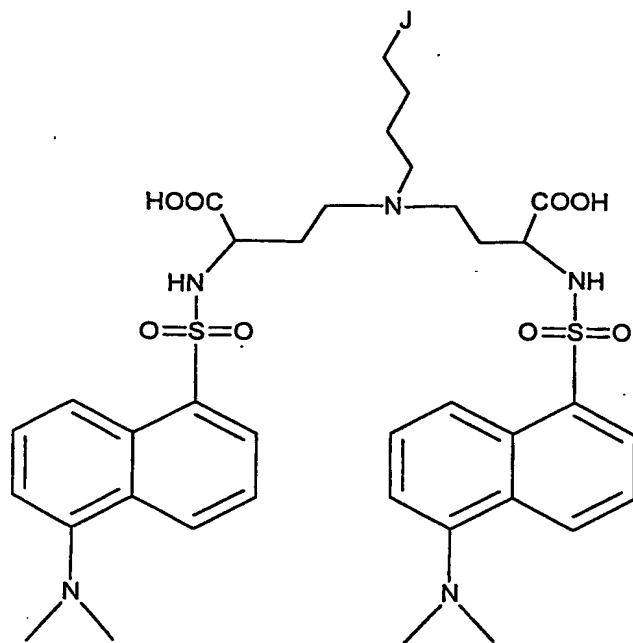
5 **16.**   The compound of Claim 15 having the following formula VII:

(VII)

wherein J is selected among –OH, -^{18}F and -Q; wherein Q is as defined in Claim 9; including pharmaceutically acceptable salts and hydrates of the compound of formula (VII).

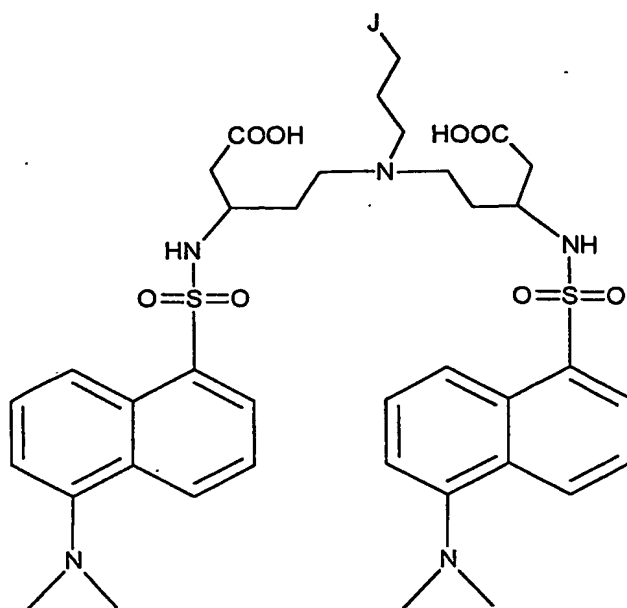**17.**   The compound of Claim 15 having the following formula VIII:

(VIII)

wherein J is selected among –OH, -^{18}F and -Q; wherein Q is as defined in Claim 9; including pharmaceutically acceptable salts and hydrates of the compound of formula (VIII).

18.   The compound of any one of Claims 17 and 18 wherein J is –OH.

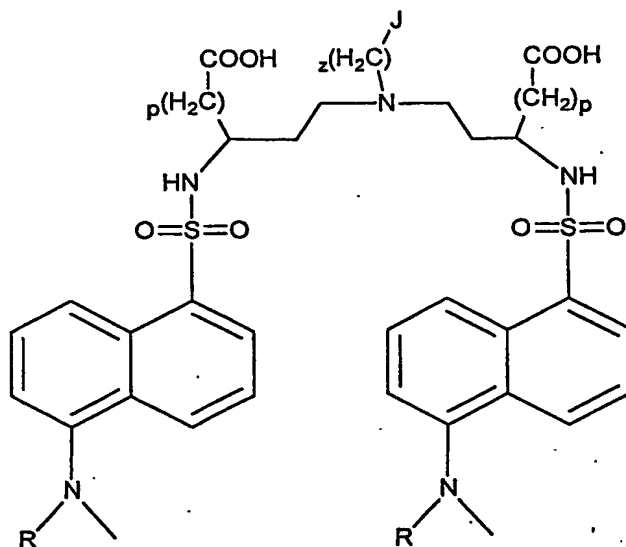**19.**   The compound of any one of Claims 17 and 18 wherein J is –^{18}F.

20. The compound of Claim 14 having the following formula IX:

(IX)

wherein J is selected among hydrogen, -OH, -^{18}F and Q; wherein Q is as defined in Claim 9; including pharmaceutically acceptable salts and hydrates of the compound of formula (IX).

21. The compound of Claim 11 having the following formula X:
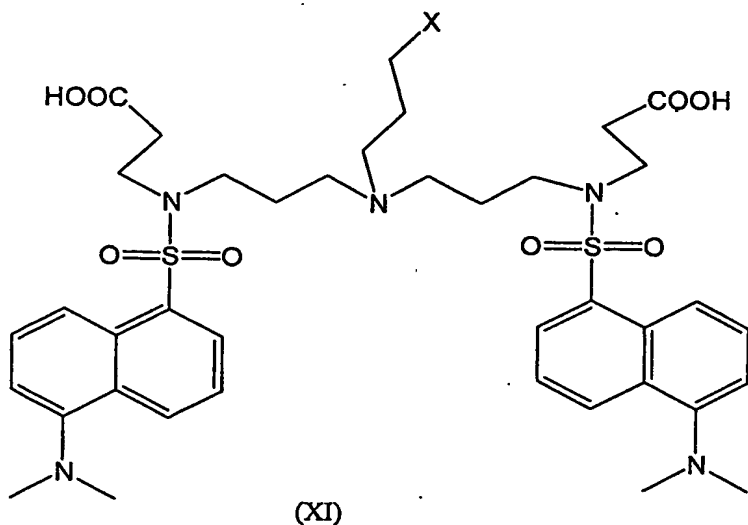

(X)

wherein p is an integer between 0-3, z is an integer between 2-6 and J is selected among –OH, ^{18}F and Q wherein Q is as defined in Claim 9;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula (X).
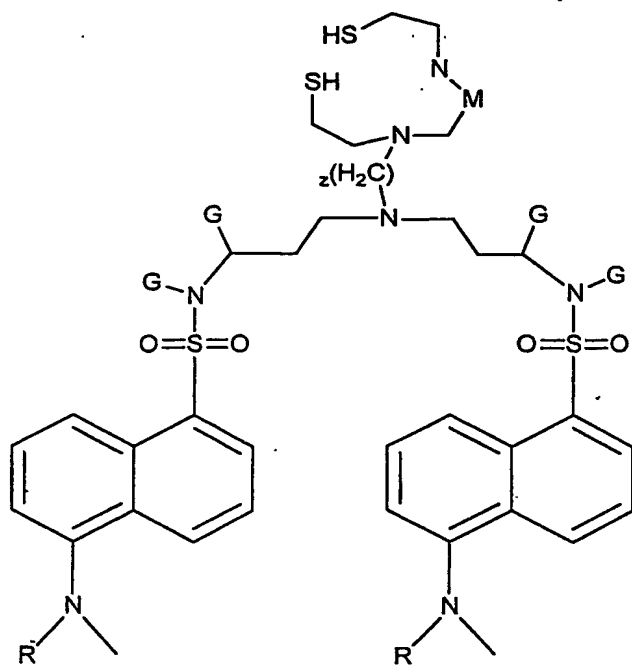
22. The compound of Claim 21 having the following formula XI:

(XI)

wherein X is selected among –OH, $-^{18}F$, $-CH_3$ and Q wherein Q is as defined in wherein Q is as defined in Claim 9;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula XI.

23. The compound of Claim 1 having the following formula XII:

(XII)

wherein M is CH_2 or $C=O$, R is a C_1-C_4 alkyl, and z is an integer of 2-6; G groups are as defined in Claim 1;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula (XII).

24.   The compound of Claim 1 having the following formula XIII:

(XIII)

wherein M is as defined in Claim 23;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula XIII.

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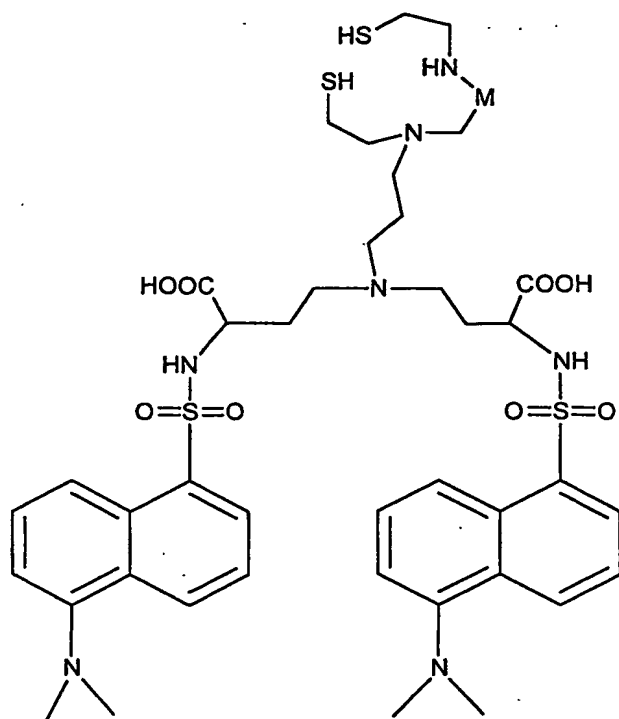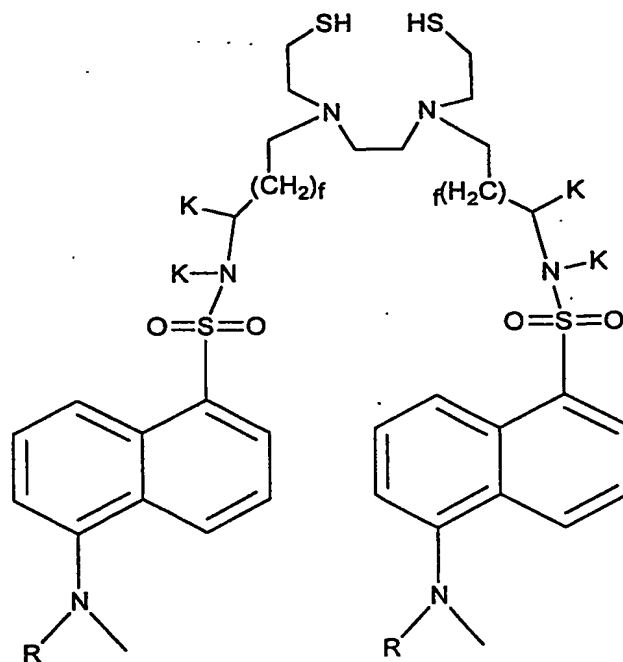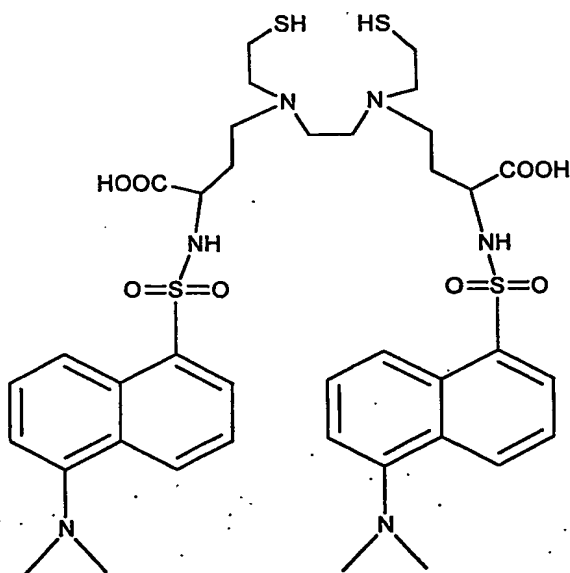25.   The compound of Claim 1 having the following formula XIV:

(XIV)

wherein f is an integer between 0-4; K is–$(CH_2)_p$-COOH, wherein p is as defined in Claim 21 and R is a C_1-C_4 alkyl;

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula (XIV)

26.   The compound of Claim 22 having the following formula XV:

(XV)

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula (XV).

27. The compound of any one of Claims 11-17, 20-26 wherein the chelated metal is selected among Technetium oxo-technetium, Rhenium and oxo-rhenium radioisotopes.

28. The compound of anyone of Claims 1 to 27 for use in the diagnosis of medical disorders in which cells undergo PNOM.

29. A diagnostic agent comprising a compound of the formula I as defined in anyone of Claims 1 to 28 and a metal, said metal being chelated through the Q moiety of the compound of Claim 1.

30. A diagnostic agent being a compound of the formula I as defined in anyone of Claims 1 to 28, wherein Q is a radioisotope.

31. A diagnostic agent being a compound of the formula I as defined in anyone of Claims 1 to 28, having fluorescence properties.

32. A diagnostic kit comprising one or more vials containing a sterile formulation comprised of a predetermined amount of a diagnostic agent according to anyone of Claims 29-31 and optionally other components, such as stabilization aids, solubilization aids or bacteriostats.

33. A diagnostic kit according to Claim 32, wherein the one or more vials that contain all or part of the formulation can independently be in the form of a sterile solution or a lyophilized solid.

34. A diagnostic composition for the detection of a perturbed membrane in a sample of biological cells, *in vitro, ex vivo, in vivo* or for clinical imaging, comprising as an active component a compound of the formula I as defined in anyone of Claims 1 to 28, together with a biologically acceptable carrier, said active component having detectable properties of its own, being capable of chelating a detectable label or being chelated to a detectable label.

35. The diagnostic composition of Claim 34, wherein said detectable label is a metal.

36.   The diagnostic composition of Claim 34, wherein the active compound has detectable properties in its own, being detected by fluorescent microscope, or by flow cytometric equipment.

37.   The diagnostic composition of Claim 34, wherein the active compound has detectable properties in its own, being detected by radioimaging techniques.

38.   The diagnostic composition of Claim 35, being a diagnostic radiocomposition for radioimaging, wherein the active compound is in the form of a metal chelate, and said metal is a radioisotope.

39.   The diagnostic composition of Claim 38, for use in single photon emission computed tomography (SPECT), wherein the metal is a radioisotope of a metal selected from Tc, In, Cu, Ga, Xe, Tl and Re.

40.   The diagnostic composition of Claim 39 wherein the metal is a radioisotope of a metal selected from Tc and Re.

41.   The diagnostic radiocomposition of Claim 40, wherein the active compound is radiolabeled with ^{99m}Tc.

42.   The diagnostic composition of Claim 34, being a diagnostic radiocomposition for radioimaging, wherein the radiolabel is a covalently linked radioisotope.

43.   The diagnostic composition of Claim 42, being a diagnostic radiocomposition for single photon emission computed tomography (SPECT).

44.   The diagnostic composition of Claim 43, wherein the radiolabel is a radioisotope of iodine.

45.   The diagnostic composition of Claim 34, being a diagnostic radiocomposition for positron emission tomography (PET), wherein the radiolabel is a covalently linked radioisotope.

46.   The diagnostic composition of Claim 45, wherein the radioisotope is selected from ^{18}F, ^{15}O, ^{18}O, ^{11}C, ^{13}C, ^{124}I, ^{13}N and ^{75}Br.

47.   The diagnostic composition of Claim 46, wherein the radioisotope is ^{18}F.

48.   The diagnostic composition of Claim 34 being a MRI contrast composition.

49. The diagnostic composition of Claim 48, wherein the active compound is in the form of a metal chelate, the metal being a paramagnetic metal ion.

50. The diagnostic composition of Claim 34 being a X-ray or computerized tomography (CT) contrast composition.

5 **51.** The agent of anyone of Claims 29-31 for use in the diagnosis of medical disorders in which cells undergo PNOM.

52. The agent of anyone of Claims 29-31 for the detection of cells undergoing a death process.

53. The agent of Claim 52 for the detection of cells undergoing apoptosis.

10 **54.** The agent of anyone of Claims 29-31 for the detection of procoagulant particles, selected among activated platelets, platelet-derived microparticles, and apoptotic bodies.

55. The agent of anyone of Claims 29- 31 for the detection of a blood clot.

56. The agent of Claims 29- 31 for the detection of activated inflammatory cells,
15 selected among activated white blood cells and activated tissue macrophages.

57. . The agent of Claim 51, for detecting the presence of a disease condition in a person already known to have the disease, for the purpose of evaluating the disease severity, monitoring disease progression, and/or monitoring a response to therapeutic modalities.

20 **58.** The agent of Claim 51, for the detection and diagnosis of a disease selected from:

diseases characterized by occurrence of excessive apoptosis, degenerative disorders, neurodegenerative disorders, Parkinson's disease, Alzheimer's disease, Huntington chorea, AIDS, myelodysplastic syndromes, ischemic or toxic insults,
25 graft cell loss during transplant rejection;

diseases manifested by excessive blood clotting; arterial or venous thrombosis, thrombo-embolism, myocardial infarction, cerebral stroke, deep vein thrombosis, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), sickle cell diseases, thalassemia,
30 antiphospholipid antibody syndrome, systemic lupus erythematosus;

inflammatory disorders, and / or diseases associated with immune–mediated etiology or pathogenesis; auto-immune disorders, antiphospholipid antibody syndrome, systemic lupus erythematosus, connective tissue disorders such as rheumatoid arthritis, scleroderma; thyroiditis; dermatological disorders, pemphigus,

5 erythema nodosum; autoimmune hematological disorders; autoimmune neurological disorders, myasthenia gravis; multiple sclerosis; inflammatory bowel disorders, ulcerative colitis; vasculitis.

59. The agent of Claim 57, wherein said detection is used to monitor adverse effects of anti-cancer treatments.

10 60. The agent of Claim 57, wherein said detection is used to monitor death of tumor cells is response to anti-cancer treatment, selected among chemotherapy and radiotherapy.

61. The agent of Claim 57, wherein said detection is used to characterize the intrinsic apoptotic load within a tumor, the level of aggressiveness of a

15 tumor, or to detect metastases.

62. The agent of Claim 57, wherein said detection is used to monitor graft survival after organ transplantation.

63. The agent of Claim 57, wherein said detection is used for diagnosis of atherosclerotic plaques.

20 64. The agent of Claim 63, wherein said detection is used for diagnosis of unstable atherosclerotic plaques.

65. The agent of Claim 57, wherein said detection is used in the monitoring of response to cytoprotective therapy in a disease characterized by excessive apoptosis, said response being inhibition of cell death.

25 66. A diagnostic kit comprising one or more vials containing a sterile formulation comprised of a predetermined amount of a diagnostic composition according to Claim 34 and optionally other components, such as stabilization aids, solubilization aids or bacteriostats.

67.   A diagnostic kit according to Claim 66, wherein the one or more vials that contain all or part of the formulation can independently be in the form of a sterile solution or a lyophilized solid.

68.   A method for the detection of cells having perturbed membranes (PM cells) in a cell sample, the method comprising:

      (i) contacting the cell sample with a diagnostic agent according to anyone of Claims 29-31 under conditions enabling binding of said agent to biological membranes; and

      (ii) detecting bound agent to said cells; the presence of a significant amount of bound agent indicating the presence of PM in said cells.

69.   A method for the detection of physiological disorders characterized by the presence of cells having perturbed membranes (PM cells), and/or medical disorders in which PM cells have an etiological or a pathogenetic role, such method comprising:

      (1) administering a diagnostic composition according to anyone of Claims 34-50 to a patient; and

      (2) imaging the patient using an appropriate imaging technique.

70.   A method according to Claim 69 wherein the diagnostic composition comprises a radiolabel, and the detection of the medical disorders is by a radioimaging technique.

71.   A method according to Claim 69 wherein the diagnostic composition comprises a radiolabel, and the detection of the medical disorders is by single photon emission computed tomography (SPECT).

72.   A method according to Claim 69 wherein the diagnostic composition comprises a radiolabel, and the detection of the medical disorders is by positron emission tomography (PET).

73.   A method according to Claim 69 wherein the diagnostic composition is an X-ray contrast agent.

74.   A method according to Claim 69 wherein the diagnostic composition comprises a magnetic resonance imaging (MRI) contrast agent.

75. A method according to Claim 69 or 68 wherein the diagnostic composition comprises a fluorescent label.

76. A method according to Claim 69 or 68, for the detection of cells undergoing a death process.

77. A method according to Claim 76, for the detection of cells undergoing apoptosis.

78. A method according to Claims 69 or 68, for the detection of procoagulant particles, selected among activated platelets, platelet-derived microparticles, and apoptotic bodies.

79. A method according to Claim 69 or 68 for the detection of a blood clot.

80. A method according to Claim 69 or 68, for the detection of activated inflammatory cells, selected among activated white blood cells and activated tissue macrophages.

81. A method according to Claim 69, for the diagnosis of a disease selected from:

diseases characterized by occurrence of excessive apoptosis; degenerative disorders, neurodegenerative disorders, Parkinson's disease, Alzheimer's disease, Huntington chorea, AIDS, myelodysplastic syndromes, ischemic or toxic insults, graft cell loss during transplant rejection;

diseases manifested by excessive blood clotting; arterial or venous thrombosis, thrombo-embolism, myocardial infarction, cerebral stroke, deep vein thrombosis, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), sickle cell diseases, thalassemia, antiphospholipid antibody syndrome, systemic lupus erythematosus; inflammatory disorders, and / or diseases associated with immune–mediated etiology or pathogenesis; auto-immune disorders, antiphospholipid antibody syndrome, systemic lupus erythematosus, connective tissue disorders such as rheumatoid arthritis, scleroderma; thyroiditis; dermatological disorders, pemphigus, erythema nodosum; autoimmune hematological disorders; autoimmune neurological

disorders, myasthenia gravis; multiple sclerosis; inflammatory bowel disorders, ulcerative colitis; vasculitis.

82. A method according to Claim 69, for the detection of atherosclerotic plaques.

83. A method according to Claim 82, for the detection of unstable atherosclerotic plaques.

84. A method according to Claim 69, for detection of cell death within a tumor, for monitoring of aggressiveness of a tumor, or for detection of metastases of a tumor.

85. A method according to Claim 69, for monitoring death of tumor cells in response to an anti-cancer treatment, selected among chemotherapy and radiotherapy.

86. A method according to Claim 69, for monitoring adverse effects of an anti-cancer treatment, wherein said adverse effects being death of normal cells.

87. A method according to Claim 69, for monitoring of survival of a grafted organ after transplantation.
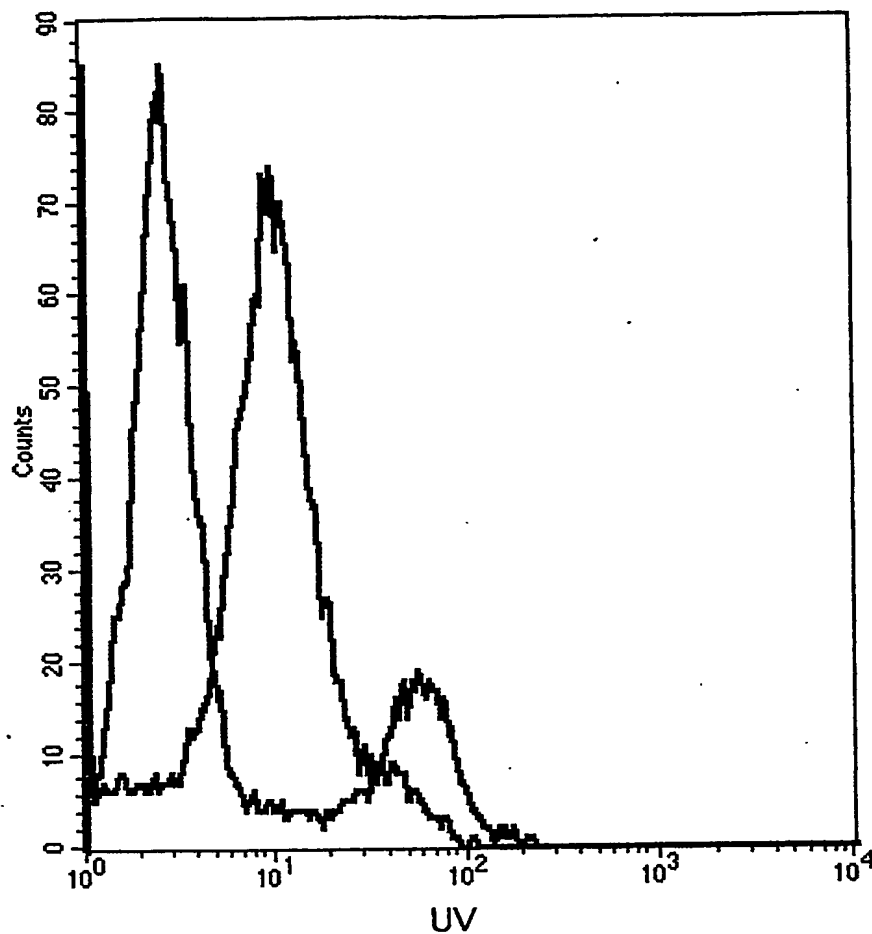
88. A method according to Claim 69, for monitoring of response to cytoprotective therapy in a disease characterized by excessive apoptosis, said response being inhibition of cell death.
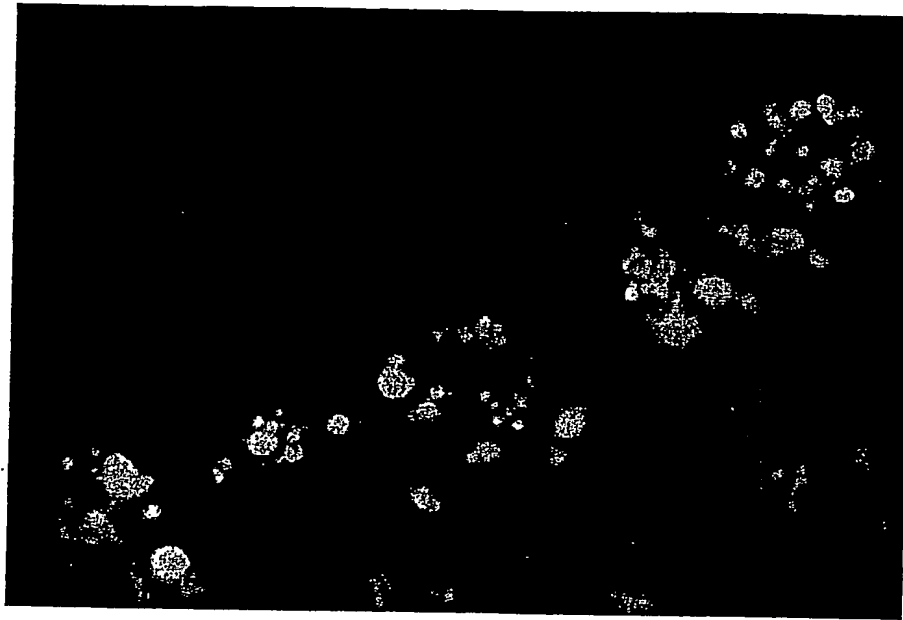
For the Applicants,
REINHOLD COHN AND PARTNERS
By: *[signature]*

Fig. 1

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Fig.2

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